



Assessment Microbiological Quality for Wild Birds Carcasses in Aswan Governorate, Egypt

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LITTLE information is available about the microbial hazards of game bird's meats. Therefore this study aimed to assess the bacteriological quality of meat cuts obtained from various species of game birds: pintail (*Anas acuta*), shoveler (*Spatula clypeata*), Eurasian wigeon (*Mareca penelope*) and Egyptian goose (*Alopochen aegyptiacus*). The mean counts of aerobic bacteria, *Enterobacteriaceae*, *Staph. aureus*, total Coliform and *E. coli* were 4.91, 4.35, 3.44, 3.94 and 3.76 in breast meat, while the corresponding counts in thigh meat were 5.19, 4.47, 3.42, 4.07 and 3.90 log CFU/g. A few counts of *Pseudomonas* and *Clostridium perfringens* (<10 CFU/g) were found, whereas yeasts and molds ranged from 10 to 2.2×10^2 CFU/g in all the examined meat samples. Aerobic bacteria, *staph. aureus*, yeasts and molds were high observed in pintail and shoveler than wigeon and Egyptian goose birds ($p < 0.05$). Neither *Salmonella* spp. nor *Listeria monocytogenes* could be isolated from the examined game bird carcasses. The public health aspects for the estimated and isolated microorganisms were discussed.

Keywords: Bacteriological Quality, Health Situation, Game bird's meats.

Introduction

Several European countries are currently observing an increase in small gamebirds harvest, but in African countries, this industry was not significantly contributing and thus it is continuing development. Game meat the greater original source of animal protein in human nutrition for poorer countries and the more developed countries, as well as game consumption was a popular activity in many communities (Ahl et al., 2002). The annual consumption of game meat in many countries differ from 0.6 to 1.0 kg per / person in Austria, France, Germany and Switzerland (Atanassova et al., 2008; Membré et al., 2011). Meanwhile, it was 3.3 kg per/person in Norway (Lillehaug et al., 2005), or in hunters' families up to 4.0 kg per/person in Northern Italy (Ramanzin et al., 2010). In many countries game meat exported or imported in substantial amount such as Germany.

Game birds may load zoonotic bacteria in their skin or intestines and transfer them to hunters through handling and cause contaminated meat. Therefore, good manufacturing practices (GMPs) throughout game bird handling and good cooling requirements of harvesting meat as specified in European union (EU) legislation at ≤ 4 °C (EC, 2004) and appropriate heat treatment are needed to reduce the health risk for hunters and consumers (Sauvala et al., 2021).

Meat from wild animals is similar to meat from domestic animals in foodborne infections, therefore, the aerobic bacterial count and *Enterobacteriaceae* count and presence or absence of pathogenic bacteria is all important factors in evaluating the sanitary quality of game meat (Atanassova et al., 2008). General indicators used in meat and poultry industries include mesophilic aerobic counts and *Enterobacteriaceae*, coliforms, *Pseudomonas* spp., lactic acid bacteria, yeasts and

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molds for evaluation of the potential shelf-life (Capita *et al.*, 2001; Álvarez-Astorga *et al.*, 2002; Capita *et al.*, 2002).

Presence of Coliforms bacteria and *E. coli* in raw meat indicates unsanitary conditions, however, they are indicators of fecal contamination at slaughter house during evisceration intestinal contents and washing (Duffy *et al.*, 2003). High level of *Enterobacteriaceae* and aerobic bacterial counts in poultry carcasses can be employed as indicators of poor hygiene during processing and storage (Roberts *et al.*, 1995; Zweifel *et al.*, 2005). Moreover, *Staph. aureus* is one of the most common food poisoning bacteriadue to the synthesis of toxins (Collins, 1967). *Pseudomonas* spp. found in everywhere and isolated from various sources, including drinking water, domestic and wild animals, humans, plants, and a variety of foods. Moreover, decrease the shelf life of food products by creating lipolytic and proteolytic enzymes, which are responsible for most of the food degradation during storage. (Arnaut-Rollier *et al.*, 1999; Adams & Maurice, 2008; Franzetti & Scarpellini, 2007). *Salmonella* spp. commonly settle in gastrointestinal tract of animals, though preventative procedures, the meat of animals contaminated with *Salmonella* during slaughter of skin or gastrointestinal tract (Arthur *et al.*, 2008; Garrido *et al.*, 2014; Wu *et al.*, 2014). Presence *Listeria monocytogenes* expected in wild avian species and also be a part of the resident microflora in food plants (Klinth-Jensen *et al.*, 2004).

In North Africa particularly Egypt, a large number of wild ducks are hunted and sold as live birds in several governorates through the period of September to March every year. Despite the presence of many studies on domestic poultry, only few studies are devoted to the microbial quality of game bird meats with only one scientific paper on wild duck meats (Khalifa & Nassar, 2001) in Egypt. Therefore, the aim of this study was to evaluate the microbiological quality of meat cuts obtained from various species of game birds namely Pintail (*Anas acuta*), Shoveler (*Spatula clypeata*), Eurasian wigeon (*Mareca Penelope*) and Egyptian Goose (*Alopochen aegyptiacus*) which are known in Egypt with the local names Balbol, Kish, Al-Sawaa and pharaony goose, respectively.

Materials and Methods

Birds

Birds were obtained in live and healthy

forms directly from hunters, where they were hunted using net instrument, and were procured of Lake Nasser hunters from Gerf Hussein south Aswan city during September and November 2020. Almost 68 birds were studied, 24 Pintail (*Anas acuta*), 24 shoveler (*Spatula clypeata*), 12 Eurasian wigeon (*Mareca Penelope*), eight Egyptian Goose (*Alopochen aegyptiacus*), each group comprised of male and female's ratio (1:1). The birds were slaughtered according to Islamic law and left to bleed freely for 5 minutes and then hand plucked and eviscerated. The carcass was cut into four quarters and washed with tap water. The pieces of meat and skin of each of the breast and thigh were minced, then packed in sterile polyethylene bags, and then stored at -20 °C until microbiological analysis was performed.

Microbiological analysis procedures

Twenty-five gm of minced breast and thigh meats was taken under antiseptic conditions. The appropriate samples were transferred to aseptic stomacher bag, mixed with 225 ml of 0.1% sterile peptone water and a homogenate in stomacher lab blender at 2000 rpm for 1-2 min to give 10⁻¹ (APHA, 1992). Serial dilutions prepared in ten-fold using sterile peptone water were spread or mixed on appropriate media for each microbe for enumeration and isolation of targeted microorganism in the present study. Samples were analyzed at Animal Health Research Institute, Food Microbiology Unit, Giza, Egypt.

Enumeration of Aerobic bacterial count

The total aerobic bacterial count of all the studied samples were determined according to the method of (ISO, 2013) as follow: One ml of three suitable serial dilutions of each sample were poured into sterile petri dish and thoroughly mixed with 15-20 ml of nutrient agar medium. Plats were incubated at (30±1) °C for (72±3) hours, then the total number of colonies was counted and multiplied by the conforming dilution to obtain the total number of bacterial colonies per gram sample. Values obtained were compared to guidelines of Egyptian organization for standardization (EOS, 2005).

Enumeration of Staph. aureus count

According to FDA (2001) using Barid-parker Agar (BPA), briefly, 1mL of required serial dilutions was poured into Barid-parker Agar plate, the plates were incubated at (37±1) °C for 24 – 48 hr. All typical colonies, characterized by gray to jet-black color with light-colored (off-white)

margin and 2-3 mm in diameter were examined and recorded as total *staph. aureus* count. Values obtained were compared to guidelines of EOS (2005).

Enumeration of coliform bacteria and E. coli

According to FDA (2002), the method was performed through three stages. First stage: Presumptive test for coliform, three tubes of lauryl tryptosesulfate (LTS) broth were inoculated with 1 ml of the serial dilutions formerly prepared 10^{-1} , 10^{-2} and 10^{-3} . The tubes were then incubated at 37°C, positive tubes produced gas, therefore, all tubes display turbidity with gas were considered as Presumptive positive for total coliforms. Second stage: Confirmatory test. Tubes containing brilliant green lactose bile (BGBL) broth were inoculated with three loopful of presumptive positive tubes and incubated at 35-37 °C for 24-48 hr. Tubes showing turbidity and gas at 37°C after 48 hr were registered as positive for total coliform. Whereas tubes display turbidity and gas production after 48 hr at 44 ± 0.2 °C were registered as positive for fecal coliform. Third stage: complementary test for *E. coli*, Chromocult Coliform Agar (CCA) plates were inoculated by positive tubes from fecal coliform and incubated for 24 hr at 37°C. All typical colonies, characterized by blue-black colonies with a green metallic sheen were specified as *E. coli*, values obtained of total coliforms were compared to guidelines of EOS (2005).

Enumeration of Enterobacteriaceae count

According to APHA (2015) using Violet Red Bile Glucose (VRBG) Agar, briefly, 1mL of required serial dilution was pour plated onto violet, red bile glucose agar, the plates were incubated at 37 ± 1 °C for 24 – 48 hr. All typical colonies, characterized by red or purple color were examined and recorded as total *Enterobacteriaceae* count; values obtained were compared to guidelines of European commission (EC, 2005).

Enumeration of Clostridium perfringens count

According to ISO (2004) using Sulfite Polymyxin Sulfadiazine (SPS) Agar medium, plates were incubated at 37 °C for 72 hr in an aerobically conditions. All typical colonies, characterized by form black colonies on SPS medium were examined and recorded as total *Clostridium perfringens* count, values obtained were compared to guidelines of EOS (2005).

Enumeration of Pseudomonas count

According to Rita Ramalho et al. (2002) using

Pseudomonas Agar Base (PAB) supplemented with CFC (Pseudomonas) Supplement contains Cetrimide, Fusidic Acid and Cefaloridin, briefly, 1mL of required serial dilution was poured plated onto PAB, plates were incubated at 41.5 °C for 24 and 48 hr.

Enumeration of yeasts and molds count

According to ISO (2008) using Dichloran Rose-Bengal Chlorotetracycline (DRBC) Agar and then the plates were incubated at 25 °C for 5-7 days; values obtained were compared to guidelines of EOS (2005).

Detection of Salmonella spp.

25 g of sample with Buffered peptone water and incubated at 34 °C and 37 °C for 24 hr (non-selective enrichment) stage, in the enrichment stage 0.1 ml of non-selective enrichment broth was transferred into Rappaport-Vassiliadis Soya (RVS) broth and incubated at 37 °C for 24 hr. From the cultures obtained enrichment stage broth inoculated with Xylose Lysine Deoxycholate (XLD) Agar and incubated at 37 °C for 24 hr, then Confirmation of *Salmonella* spp. was made using biochemical tests according to ISO (2017a).

Detection of Listeria monocytogenes

25 g of sample with Buffered Listeria enrichment broth (BLEB) and incubated at 30 °C for 24 hr to 26 hr (selective enrichment) stage, in the isolation stage take one loop full of the enriched broth using sterile inoculating loop and streak on Oxford Agar (OXA) and incubated at 35 °C for 24-48 hr, then after incubation typical Listeria species colonies are gray to black colonies surrounded by a black halo (ISO, 2017b).

Statistical analysis

Results were expressed as the mean \pm standard error. Statistical differences between genotype and sexes were specified by tow-way analysis of variance (ANOVA) using the Duncan's Multiple Range test at significant level of 0.05 using SPSS program (SPSS, 2001). for windows software, version 25. The significance difference was set at ($p < 0.05$).

Results and Discussion

Microbial analysis of meat samples taken from breast and thigh of wild birds (male and female) was carried out and the obtained results were tabulated in Tables 1 & 2. Data revealed that total of aerobic bacterial count ranged between 3.98 – 5.80 and 4.28 – 5.90 CFU/g for meat samples of breast and thigh, respectively. It could be concluded that

there was a significant difference in total aerobic bacterial count between the different birds from breast and leg meats at ($P < 0.05$). Therefore, the higher aerobic bacterial count was observed in breast of Pintail and Shoveler birds (5.46 to 5.80 log CFU/g), while the lower count was in breast of wigeon and Egyptian goose birds (3.96 to 4.47 log CFU/g) (Table 1). The same trend was observed in thigh muscles, where aerobic bacterial count recorded (5.72 to 5.90 log CFU/g) and (4.28 to 5.02 log CFU/g) for Pintail, Shoveler birds and wigeon, Egyptian goose birds, respectively. These findings agree with that recorded by Abdallaha *et al.* (2014) which calculated the total count of aerobic bacteria in domestic duck carcasses in Egypt by about 4.97 and 5.49 CFU/g for breast and thigh meat, respectively. Also, El-Ghareeb *et al.* (2009) determined the aerobic bacterial count in breast and thigh meat of wild partridges, quails and pheasants by about (4.19 and 5.30), (6.48); (5.48 and 5.48) log CFU/g, respectively. However, it was lower compared to the findings obtained by Edris *et al.* (2015) in chicken meat 7.94 log CFU/g. According to the safe permissible limit specified by EOS (2005) for total aerobic bacterial count, it was clear that, the result of Pintail and Shoveler meats was not compatible to EOS that recommended aerobic bacterial counts not exceed 10^5 , whilst wigeon and Egyptian goose meats agree with EOS limits. The higher microbial load of examined wild bird carcasses samples may be accredited to those live birds loading great numbers of bacteria on their faces, feet, and feathers. In addition to cross contamination through scalding and defeathering processes and the unsanitary hygienic conditions through handling particularly during evisceration which play a main role in microbial contamination.

Numbers of *Staph. aureus* generally ranged from <10 CFU/g to 3.69 log CFU/g in breast and thigh meats. On the other hand, there was a highly significant difference of *Staph. aureus* between the different birds from breast and thigh meats at ($P < 0.05$). Results in Tables 1 & 2, revealed that the higher count was in breast and thigh meat of Pintail and Shoveler birds 3.23 to 3.69 log CFU/g, while the lower count was recorded in breast and thigh meat of wigeon and Egyptian goose birds <10 CFU/g except breast meat of Egyptian goose male recorded 3.03 log CFU/g. These results came in accordance with those of other investigators in duck carcasses 3.34 log CFU/g (Abdallaha *et al.*, 2014), in wild birds <2.00 to 5.90 log CFU/g (El-

Ghareeb *et al.*, 2009) and in wild quail 3.30 log CFU/g (El-Dengawy & Nassar, 2001), while higher count reported by Buzón-Durán *et al.* (2017) in raw poultry-based meat preparations 4.07 log CFU/g. According to the safe permissible limit specified by EOS (2005) recommended that, *Staph. aureus* (didn't exceed 10^2 cfu/g), therefore, samples of Pintail and Shoveler birds were unaccepted (exceeded >2.00 log CFU/g), but wigeon and Egyptian goose bird samples were accepted (didn't exceed <2.00 log CFU/g). The highest count of *Staph. aureus* may be due to sample meat minced with skin and the presence of *Staph. aureus* in a food marker contamination from workers handling, skin and nose or mouth. The wide variation in *Staph. aureus* counts between different bird meats may be attributed to environmental conditions for birds, feeding variation (herbivorous, carnivorous, and omnivorous) and potential differences in handling by personnel.

Presence of Coliforms and *E. coli* in raw meat was due to serious deficiencies in sanitary conditions, however, they are indicators of fecal contamination at slaughter house during evisceration "intestinal contents" and washing (Duffy *et al.*, 2003). The coliforms and *E. coli* counts were achieved in Tables 1 & 2. The results revealed that *E. coli* and Coliforms ranged from 2.95 to 4.48 and 3.57 to 4.84 log CFU/g in breast and thigh meats, respectively. Moreover, it was clear that, there was no significant difference of Coliforms and *E. coli* counts between the different birds from breast and thigh meats at ($P > 0.05$). The present results are also in agreement with values observed by El-Ghareeb *et al.* (2009) in wild partridges, pigeons, quails and pheasants in Egypt *E. coli* recorded 4.12 log CFU/g and Paulsen *et al.*, (2008) in uneviscerated wild pheasant in the Slovak Republic *E. coli* recorded 2.0 to 4.1 log CFU/g, in contrast, lower coliforms loads were observed by El-Dengawy & Nassar (2001) for wild quail carcasses in Egypt 6×10^2 CFU/g and Cordero *et al.* (2019) for domestic and wild pigeon breast meats in Spain 2.11 and 1.10 log CFU/g, respectively. According to the safe permissible limit specified by EOS (2005) recommended that, coliforms (didn't exceed 10^2 CFU/g), therefore, samples of all examined muscles were unaccepted (exceeded >2.00 log CFU/g). The contamination with Coliforms may be from water as sources of Coliforms in meat and during slaughtering and dressing of carcasses and cutting carcasses into various parts. Moreover, the presence of *E. coli* in the examined samples is an indicator for carcass may be contaminated with intestinal microflora of animals.

TABLE 1. Microbial counts of breast meat samples from pintail and shoveler, Eurasian wigeon, Egyptian geese (Mean± standard error).

Microorganisms	Northern Pintail		Northern Shoveler		Eurasian wigeon		Egyptian Geese		P-Value	
	Male	Female	Male	Female	Male	Female	Male	Female	Genotype (G)	Sex (S)
	Aerobic bacterial count	5.8 ± 0.2	5.7 ± 0.2	5.6 ± 0.6	5.46 ± 0.3	3.98 ± 0.5	4.31 ± 0.2	4.47 ± 0.3	3.96 ± 0.12	0.001
<i>Staph. aureus</i>	3.51 ± 0.6	3.53 ± 0.3	3.47 ± 0.3	3.23 ± 0.6	<10	<10	3.03 ± 0.3	<10	0.000	0.425
<i>Escherichia coli</i>	3.92 ± 0.1	4.84 ± 0.5	3.90 ± 0.8	3.27 ± 0.7	3.63 ± 0.3	3.01 ± 0.4	3.68 ± 0.2	3.83 ± 0.5	0.193	0.896
Total coliform	4.03 ± 0.1	4.6 ± 0.8	3.99 ± 0.3	3.48 ± 0.6	4.03 ± 0.4	3.51 ± 0.4	3.89 ± 0.0	4 ± 0.7	0.649	0.815
<i>Enterobacteriaceae</i>	4.08 ± 0.3	4.91 ± 0.5	4.30 ± 0.6	3.70 ± 0.4	4.68 ± 0.1	3.74 ± 0.2	4.81 ± 0.8	4.60 ± 0.4	0.455	0.493
<i>Clostridium perfringens</i>	2.1	2.27	<10	<10	<10	<10	<10	<10	-	-
<i>Pseudomonas</i>	<10	<10	<10	<10	<10	<10	<10	<10	-	-
Yeasts	2.1 × 10 ¹	3 × 10	2.2 × 10 ²	<10	<10	<10	<10	<10		
Molds	7 × 10	1 × 10 ²	7 × 10	6 × 10	<10	<10	<10	<10		
<i>Salmonella</i> spp.	ND	ND	ND	ND	ND	ND	ND	ND		
<i>Listeria monocytogenes</i>	ND	ND	ND	ND	ND	ND	ND	ND		

Abbreviations: **log (CFU/g)** log colony forming units per gram (CFU/g), <10 means lower count than colony forming units per gram (CFU/g), (ND) No detected, Yeast and Molds calculated as (CFU/g), (p-value) of > 0.05 non-significant (NS) difference, p-value < 0.05 significant difference.

TABLE 2. Microbial counts of thigh meat samples from pintail and shoveler, Eurasian wigeon, Egyptian geese leg meats. (Mean± standard error).

Microorganisms	Northern Pintail		Northern Shoveler		Eurasian wigeon		Egyptian Geese		P-Value	
	Male	Female	Male	Female	Male	Female	Male	Female	Genotype (G)	Sex (S)
Aerobic bacterial count										
<i>Staph. aureus</i>	5.79±0.2	5.72±0.1	5.73±0.7	5.90±0.1	4.28±0.6	4.70±0.5	5.02±0.1	4.41±0.5	0.008	0.946
<i>Escherichia coli</i>	3.3±0.4	3.69±0.2	3.39±0.4	3.30±0.6	<10	<10	<10	<10	0.000	0.720
Total coliform	3.89±0.6	4.76±0.3	4.02±0.6	4.24±0.3	3.09±0.3	4.31±0.4	3.96±0.6	2.95±0.9	0.376	0.399
<i>Enterobacteriaceae</i>	3.95±0.6	3.85±0.5	4.06±0.1	4.28±0.3	3.57±0.2	4.43±0.4	4.48±0.4	3.91±0.6	0.881	0.731
<i>Clostridium perfringens</i>	4.07±0.2	4.89±0.5	4.87±0.3	4.28±0.7	3.76±0.8	4.38±0.1	5.57±0.5	3.95±0.9	0.676	0.644
<i>Pseudomonas</i>	<10	<10	<10	<10	<10	<10	<10	<10	-	-
Yeasts	<10	<10	<10	<10	<10	<10	<10	<10	-	-
Molds	1.7×10 ³	10×3	2.1×10 ²	5×10	<10	<10	1×10	1×10		
<i>Salmonellaspp.</i>	1×10	7×10	7×10	7×10	<10	<10	1×10	1×10		
<i>Listeria monocytogenes</i>	ND	ND	ND	ND	ND	ND	ND	ND		
	ND	ND	ND	ND	ND	ND	ND	ND		

Abbreviations: log (CFU/g) log colony forming units per gram (CFU/g), <10 means lower count than colony forming units per gram (CFU/g), (ND) No detected, Yeast and Molds calculated as (CFU/g), (p-value) of > 0.05 non-significant (NS) difference, p-value < 0.05 significant difference.

Total plate count of *Enterobacteriaceae* especially *E. coli* are generally used as indicator for deficiencies in hygiene programs according to EC (2005). These bacteria may occur during unsatisfactory hygienic measures of handling, processing, and storage. The *Enterobacteriaceae* counts of wild birds' meat are presented in Tables 1 & 2, The results revealed that, *Enterobacteriaceae* ranged from 3.70 to 4.91 and 3.76 to 5.57 log CFU/g in breast and thigh meats, respectively. No significant differences were observed on *Enterobacteriaceae* counts between the different birds from breast and thigh meats at ($P > 0.05$). The number of *Enterobacteriaceae* isolated from wild birds' meat in the present study was almost like that obtained by previous investigators.

El-Ghareeb et al. (2009) reported that the number of *Enterobacteriaceae* isolated from fresh breast and thigh muscles of hunted partridges and pigeons were (4.00; 4.48) and (3.48; 4.00) log CFU/g, respectively. The same value was recorded by Mousa et al. (2016). Also, Abdallah et al. (2014) found that the mean values of total *Enterobacteriaceae* isolated from frozen breast and thigh duck meat were 7.85×10^3 and 9.13×10^4 CFU/g, respectively. On the other hand, lower values (2.60 to 3.74 log CFU/g) were found in breast, thigh and liver cuts of farm and hunted wild pheasants (Buřková et al., 2016). Also, 2.06 and 1.35 log CFU/g were recorded by Cordero et al. (2019) for breast meat of domestic and wild pigeon, respectively. According to the safe permissible limit specified by Center for Food Safety (CFS, 2014), which recommended that, *Enterobacteriaceae* should not exceed (10^2 CFU/g), and also EC (2005) which recommended that, *Enterobacteriaceae* should not exceed 2,5 log CFU/ cm², therefore, samples of all samples examined in the current study were unaccepted (exceeded >2.00 log CFU/g).

Pseudomonas species are linked with spoilage of meat producing off-odors, off-flavors, discoloration and gas production (Borch et al., 1996; Arnaut-Rollier et al., 1999). More recent, Franzetti & Scarpellini (2007) reported that *Pseudomonas* spp. reduce the shelf life and thus the quality of food products by releasing lipolytic and proteolytic enzymes, which are the most common causes of food spoilage during storage.

However, examination of breast and thigh meat samples of the studied wild birds for the

presence of *Pseudomonas* bacteria indicated that total counts of *Pseudomonas* spp. were <10 log CFU /g of all examined samples, as shown in Tables 1 & 2. According to Mead (2005), the initial count of *Pseudomonas* species on poultry products should not exceed 100 CFU/g under aerobic conditions to provide optimal storage life and sensory qualities. Therefore, *Pseudomonas* count obtained in the present study was lower than the recommended limits.

Poultry carcass and meat cuts may be contaminated with *Clostridium perfringens* from intestinal contents through slaughter house process especially during evisceration (Voidarou et al., 2011). Thus, numbers of *Clostridium Perfringens* in the present study were found to be <10 CFU/g for all examined muscles or may not be exist, as shown in Tables 1 & 2, except breast muscles of Pintail male and females recorded 2.1 and 2.27 log CFU/g. Refer to some previous studies, (Khalifa & Nassar, 2001; El-Dengawy & Nassar, 2001) it found that, *Clostridium perfringens* could not be detected in breast and thigh of wild duck and quails, but in other studies Shaltout et al. (2017) found that total count of vegetative form of *Clostridium perfringens* in raw and cooked chicken meat samples were 1.15×10^4 and 2.7×10^2 CFU/g, respectively. Shaltout et al. (2017) found that, total count of vegetative form of *Clostridium perfringens* in raw breast and thigh chicken meat samples were 9.1×10^3 and 2.5×10^4 CFU/g, respectively. According to the safe permissible limit specified by EOS (2005) recommended that, *Clostridium Perfringens* (didn't exceed 10^3 CFU/g).

Regarding mold and yeast counts in tested wild birds' meat samples data in Tables 1&2 revealed that, pintail and shoveler samples showed higher contamination levels than wigeon and Egyptian goose samples. The breast samples of pintail and shoveler recorded 30 to 220 and 60 to 100 CFU/g for yeasts and molds, respectively. Leg samples of the same two types of birds contained 30 to 210 CFU/g for yeasts and 10 to 70 CFU/g for molds. In contrast, the contamination levels of wigeon and Egyptian goose samples with yeasts or molds recorded <10 CFU/g in all examined samples. The present results were consistent with that observed by Mahdy et al. (2019) (10 to 1.1×10^3 CFU/g) in chilled and frozen duck meat, and by Odetunde et al. (2011) (1.3×10^1 to 1.5×10^2 CFU/g in chilled chicken meat. Meanwhile, Abdallaha et al. (2014) recorded 7.57×10^2 and 1.12×10^3 CFU/g in duck

breast and thigh meat. In contrast, Almorshidy (2013) found that, raw poultry samples were free from fungi. According to the maximum permissible limits stipulated by EOS (2005) which recommended that the contamination level should not be exceed than 10^2 CFU/g. On the other hand, most of the studied samples especially of wigeon and Egyptian goose proved to be acceptable.

Salmonella spp. and *Listeria monocytogenes* were not detected in all examined samples. it is evident that the *Salmonella* Spp. are in the permissible limit of (EOS, 2005) for raw poultry meat (sample should be free), so that, these samples are accepted according to the EOS (2005). The obtained results are similar to that obtained by Sidorov et al. (2021) they found that, *Salmonella* was not detected in wild wigeon, shoveler and scoter duck carcasses samples, except for mallard duck carcass. Also, Paulsen et al. (2008) and El-Dengawy & Nassar (2001) found that, *salmonella* was not recovered from any sample of wild pheasant and wild quail carcasses in Egypt.

Conclusion

From the presented results it could be concluded that birds' meats were highly contaminated with *Enterobacteriaceae*, Coliform and *E. coli*, therefore, this indicates that higher microbial load in wild birds may be accredited to those live birds loading great numbers of bacteria on their faces, feet, feathers, and skin, as well as lack of hygiene measures during handling and processing by hunters. Wigeon and Egyptian goose birds showed safe for human consumption than pintail and shoveler birds, because its lower count of aerobic bacterial count, *Staphylococcus auras*, molds and yeasts. Therefore, there is an urgent need to improve health situation of wild bird game meats, hence, good manufacturing practices (GMPs) during game bird handling, good cooling requirements, appropriate heat treatment (cooking) and E-beam irradiation applications must be enforced to reduce microbial load.

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References

- Abdallaha, R. N., Hassanen, F. S., Salem, A. M., and El-Shater, M. A. (2014) Bacterial evaluation of frozen cut-up duck meat. *Benha Veterinary Medical Journal*, **26**(2), 30-39. <https://www.bvmj.bu.edu.eg/issues/26-2/4.pdf>
- Adams, M.R. and Maurice, O.M. (2008) Food Microbiology. Royal Society of Chemistry. Cambridge. GB. 2008. p.463
- Ahl, A.S., Nganwa, D. and Wilson, S. (2002) Public Health Considerations in Human Consumption of Wild Game. *Annals of the New York Academy of Sciences*, **969**(1): 48–50. <https://doi.org/10.1111/j.1749-6632.2002.tb04349.x>
- Almorshidy, O.E. (2013) Aflatoxin content of raw and processed poultry meat products sold in Alexandria markets. *Master thesis*, Faculty of Veterinary Medicine, Alexandria University.
- Álvarez-Astorga, M., Capita, R., Alonso-Calleja, C., Moreno, B. and García-Fernández, C. (2002) Microbiological quality of retail chicken by-products in Spain. *Meat science*, **62**(1), 45-50. [https://doi.org/10.1016/s0309-1740\(01\)00225-x](https://doi.org/10.1016/s0309-1740(01)00225-x)
- APHA (1992) “American Public Health Association” Compendium of methods for microbiological examination of Food. <https://doi.org/10.2105/MBEF.0222>
- APHA (2015) “American Public Health Association” Standard Methods for the Examination of Water and Wastewater (23rd edition), Washington D.C.
- Arnaut-Rollier, I., Vauterin, L., De Vos, P., Massart, D. L., Devriese, L. A., De Zutter, L. and Van Hoof, J. (1999) A numerical taxonomic study of the *pseudomonas* flora isolated from poultry meat. *Journal of Applied Microbiology*, **87**(1), 15-28. <https://doi.org/10.1046/j.1365-2672.1999.00785.x>
- Arthur, T. M., Brichta-Harhay, D. M., Bosilevac, J. M., Guerini, M. N., Kalchayanand, N., Wells, J. E., Shackelford, S.D., Wheeler, T.L. and Koohmaraie, M. (2008) Prevalence and Characterization of *Salmonella* in Bovine Lymph Nodes Potentially Destined for use in Ground Beef. *Journal of Food Protection*, **71**(8), 1685-1688. <https://doi.org/10.4315/0362-028X-71.8.1685>
- Atanassova, V., Apelt, J., Reich, F. and Klein, G. (2008) Microbiological Quality of Freshly Shot Game in Germany. *Meat Science*, **78**(4):), 414–19. <https://doi.org/10.1016/j.meatsci.2007.07.004>

- Borch, E., Kant-Muermans, M. L. and Blixt, Y. (1996) Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology*, **33**(1), 103-120. [https://doi.org/10.1016/0168-1605\(96\)01135-X](https://doi.org/10.1016/0168-1605(96)01135-X)
- Buňková, L., Gál, R., Lorencová, E., Jančová, P., Doležalová, M., Kmeř, V. and Buňka, F. (2016) Microflora of farm and hunted pheasants in relation to biogenic amines production. *European Journal of Wildlife Research*, **62**(3), 341-352. <https://doi.org/10.1007/s10344-016-1008-y>
- Buzón-Durán, L., Capita, R. and Alonso-Calleja, C. (2017) Microbial loads and antibiotic resistance patterns of *Staphylococcus aureus* in different types of raw poultry-based meat preparations. *Poultry Science*, **96**(11), 4046-4052. <https://doi.org/10.3382/ps/pex200>
- Capita, R., Alonso-Calleja, C., Garcia-Fernandez, M.D.C. and Moreno, B. (2001) Microbiological quality of retail poultry carcasses in Spain. *Journal of Food Protection*, **64**(12), 1961-1966. <https://doi.org/10.4315/0362-028X-64.12.1961>
- CFS (2014) "Center for Food Safety: Microbiological guidelines for food (for ready to-eat food in general and specific food items). In Food and Environmental Hygiene Department, Hong Kong. https://www.cfs.gov.hk/english/food_leg/files/food_leg_Microbiological_Guidelines_for_Food_e.pdf
- Collins, C.H. (1967) Microbiological methods, (2nd Edition), Published by Plenum Press, New York. https://mmstcchemistry.weebly.com/uploads/2/4/1/2/24121933/microbiological_methods.pdf
- Cordero, J., Alonso-Calleja, C., García-Fernández, C. and Capita, R. (2019) Microbial load and antibiotic resistance patterns of *Escherichia coli* and *Enterococcus faecalis* isolates from the meat of wild and domestic pigeons. *Foods*, **8**(11), 536. <https://doi.org/10.3390/foods8110536>
- Duffy, G., Cagney, C., Crowley, H. and Sheridan, J.J. (2003) A Nationwide Surveillance Study on *E. coli* O157: H7 and *Enterobacteriaceae* in Irish Minced Beef Products. Teagasc. <https://t-stor.teagasc.ie/bitstream/handle/11019/148/Report%2063.pdf?sequence=1&isAllowed=y>
- EC (2004) "Europun Commission Regulation" No 853/2004 of the European parliament and of the council of 29 April 2004 laying down specific hygiene rules for food of animal origin. *Journal of the European Union*, **139**, 55-205. <https://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:139:0055:0205:en:PDF>
- EC (2005) "Europun Commission Regulation" No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Journal of the European Union*, **50**, 1-26. <https://eur-lex.europa.eu/eli/reg/2005/2073/oj>
- Edris, A.M., Amin, R. A., Nassif, M. Z. and Mahmoud, M. Z. (2015) Bacterial status of fresh marketed chicken meat cuts-up. *Benha veterinary Medical Journal*, **28**(2), 52-57. <https://doi.org/10.21608/BVMJ.2015.31863>
- El-Dengawy, R.A. and Nassar, A.M. (2001) Investigation on the nutritive value and microbiological quality of wild quail carcasses. *Food/Nahrung*, **45**(1), 50-54. [https://doi.org/10.1002/1521-3803\(20010101\)45:1<50::AID-FOOD50>3.0.CO;2-J](https://doi.org/10.1002/1521-3803(20010101)45:1<50::AID-FOOD50>3.0.CO;2-J)
- El-Ghareeb, W.R., Smulders, F.J.M., Morshdy, A.M.A., Winkelmayr, R. and Paulsen, P. (2009) Microbiological condition and shelf life of meat from hunted game birds. *European Journal of Wildlife Research*, **55**(4), 317-323. <https://doi.org/10.1007/s10344-009-0249-4>
- EOS (2005) "Egyptian Organization for Standardization" (1651/2005). Chilled poultry and rabbit.
- FDA (2001) "Food and Drug Administration" US. Department of health and human services. *Public Health Service*. College Park, MD 20740.
- FDA (2002) "Food and Drug Administration" Chapter No. 4: Enumeration of *Escherichia coli* and the Coliform Bacteria. *Bacteriological Analytical Manual*, U.S. <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria>
- Franzetti, L. and Scarpellini, M. (2007) Characterisation of *Pseudomonas* spp. isolated from foods. *Annals of microbiology*, **57**(1), 39-47. <https://doi.org/10.1007/BF03175048>
- Garrido, V., Sánchez, S., San Román, B., Zabalza-Baranguá, A., Díaz-Tendero, Y., de Frutos, C., Mainar-Jaime, R. and Grilló, M. J. (2014) Simultaneous Infections by Different Salmonella strains in Mesenteric lymph Nodes of Finishing Pigs. *BMC Veterinary Research*, **10**(1), 1-6. <https://doi.org/10.1186/1746-6148-10-59>
- ISO (2004) "International Standards Organization" 7937 Microbiology of food and animal feeding *Egypt. J. Food Sci.* **51**, No. 1 (2023)

- stuffs – horizontal method for the enumeration of *Clostridium perfringens*– Colony count technique. <http://www.oxid.com/pdf/iso-food-safety-brochure.pdf>
- ISO (2008) “International Standards Organization” 21527-2. Microbiology of food and animal feeding stuffs —Horizontal method for the enumeration of yeasts and moulds —Part 2: Colony count technique. <http://www.oxid.com/pdf/iso-food-safety-brochure.pdf>
- ISO (2013) “International Standards Organization” 4833-1. Microbiology of food chain- Horizontal method for the enumeration of microorganisms. Part I; Colony count at 30 C by the pour plate technique. International Standards Organization, Geneva, Switzerland. <http://www.oxid.com/pdf/iso-food-safety-brochure.pdf>
- ISO (2017a) “International Standards Organization” 4833-16579-1. Microbiology of the Food Chain—Horizontal Method for the Detection, Enumeration and Serotyping of *Salmonella*—Part 1: Detection of *Salmonella* Spp.; International Organisation for Standardisation: Geneva, Switzerland, 2017. <https://www.iso.org/standard/56712.html>
- ISO (2017b) “International Standards Organization” 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method. <https://www.iso.org/standard/60313.html>
- Khalifa, A.H. and Nassar, A.M. (2001) Nutritional and bacteriological properties of some game duck carcasses. *Food/Nahrung*, **45**(4), 286-292. [https://doi.org/10.1002/1521-3803\(20010801\)45:4<286::AID-FOOD286>3.0.CO;2-%23](https://doi.org/10.1002/1521-3803(20010801)45:4<286::AID-FOOD286>3.0.CO;2-%23)
- Klinth-Jensen, W., Devine, C. and Dikeman, M. (2004) *Encyclopedia of Meat Sciences*. Oxford, UK: Elsevir, 396-402..
- Lillehaug, A., Bergsjø, B., Schau, J., Bruheim, T., Vikøren, T. and Handeland, K. (2005) *Campylobacter* spp., *Salmonella* spp., verocytotoxic *Escherichia coli*, and antibiotic resistance in indicator organisms in wild cervids. *Acta Veterinaria Scandinavica*, **46**(1), 1-10. <https://doi.org/10.1186/1751-0147-46-23>
- Mahdy, A., Salem, A. and Zaghoul, M. (2019) Mycological Assessment of Marketed Duck Meat in El-Kaliobia Governorate Markets. *Benha Veterinary Medical Journal*, **37**(1), 69-72. <https://doi.org/10.21608/BVMJ.2019.17864.1108>
- Mead, G. C. (2005). Food safety control in the poultry industry. CRC Press. Woodhead Pub., Boca Raton, FL, Cambridge.
- Membré, J.M., Laroche, M. and Magras, C. (2011) Assessment of Levels of Bacterial Contamination of Large Wild Game Meat in Europe. *Food Microbiology*, **28**(5): 1072–79. <https://doi.org/10.1016/j.fm.2011.02.015>
- Mousa, M.M., Salem, N.I., Khalifa, A.M., Abd-El-Hady, H.A., and El Gamel, A.M. (2016). Quality assessment of frozen quail in Kafr El-Sheikh Governorate. *Alexandria Journal of Veterinary Sciences*, **48**, 57-61. <https://doi.org/10.5455/ajvs.180181>
- Odetunde, S., Lawal, A.K., Akolade, M. and Bakry, S. (2011) Microbial flora of frozen chicken part varieties. *International Research Journal of Microbiology*, **2**(11), 423-427. <https://www.interesjournals.org/articles/microbial-flora-of-frozen-chicken-part-varieties.pdf>
- Paulsen, P., Nagy, J., Popelka, P., Ledecy, V., Marcinčák, S., Pipova, M., Smulders, F.J.M., Hofbauer, P., Lazar, P. and Dı́cakova, Z. (2008) Influence of storage conditions and shotshell wounding on the hygienic condition of hunted, unviscerated pheasant (*Phasianus colchicus*). *Poultry Science*, **87**(1), 191-195. <https://doi.org/10.3382/ps.2007-00039>
- Ramanzin, M., Amici, A., Casoli, C., Esposito, L., Lupi, P., Marsico, G., Mattiello, S., Olivieri, O., Ponzetta, M.P., Russo, C. and Marinucci, M. T. (2010) Meat from wild ungulates: ensuring quality and hygiene of an increasing resource. *Italian Journal of Animal Science*, **9**(3), e61. <https://doi.org/10.4081/ijas.2010.e61>
- Ramalho, R., Cunha, J., Teixeira, P. and Gibbs, P. A. (2002) Modified Pseudomonas agar: new differential medium for the detection/enumeration of *Pseudomonas aeruginosa* in mineral water. *Journal of Microbiological Methods*, **49**(1), 69-74. [https://doi.org/10.1016/S0167-7012\(01\)00365-7](https://doi.org/10.1016/S0167-7012(01)00365-7)
- Roberts, D., Hooper, W. and Greenwood, M., (1995) Practical Food Microbiology: Methods for the Examination of Food for Micro-Organisms of Public Health Significance. Public Health Laboratory Service.
- Sauvala, M., Woivalin, E., Kivistö, R., Laukkanen-Ninios, R., Laaksonen, S., Stephan, R. and Fredriksson-Ahomaa, M. (2021) Hunted game birds—Carriers of foodborne pathogens. *Food Microbiology*, **98**, 103768. <https://doi.org/10.1016/j.fm.2021.103768>

- Shaltout, F.A., Zakaria, I.M. and Nabil, M.E. (2018) Incidence of some anaerobic bacteria isolated from chicken meat products with special reference to *Clostridium perfringens*. *Benha Veterinary Medical Journal*, **2**, 429-438. <https://doi.org/10.21608/BVMJ.2017.30489>
- Shaltout, F.A., Osman, I.M., Kamel, E.A. and Abd-Alla, A.K. (2017) Isolation of *Clostridium perfringens* from Meat Samples Obtained from the University Students' Hostel. *EC Nutrition*, **9**(3), 142-150. file:///C:/Users/AS/Downloads/Isolation_of_Clostridium_perfringens_fro.pdf
- Sidorov, M.N., Tomashevskaya, E.P. and Maksimova, A.N. (2021) Veterinary-Sanitary Examination of Migratory Bird Carcasses When Stored in a Glacier Under Permafrost Conditions. In IOP Conference Series: Earth and Environmental Science (Vol. 666, No. 2, p. 022057). IOP Publishing.
- SPSS (2001) Statistical Packages for the Social Sciences. Statistical software for windows version 25. Micro -soft, Chicago, IL, USA.
- Voidarou, C., Vassos, D., Rozos, G., Alexopoulos, A., Plessas, S., Tsinas, A., Skoufou, M., Stavropoulou, E., Bezirtzoglou, E. and Bezirtzoglou, E. (2011) Microbial challenges of poultry meat production. *Anaerobe*, **17**(6), 341-343. <https://doi.org/10.1016/j.anaerobe.2011.05.018>
- Wu, D., Alali, W.Q., Harrison, M.A. and Hofacre, C.L. (2014) Prevalence of Salmonella in Neck Skin and Bone of Chickens. *Journal of Food Protection*, **77**(7), 1193-1197. <https://doi.org/10.4315/0362-028X.JFP-14-006>
- Zweifel, C., Baltzer, D. and Stephan, R. (2005) Microbiological contamination of cattle and pig carcasses at five abattoirs determined by swab sampling in accordance with EU Decision 2001/471/EC. *Meat Science*, **69**(3), 559-566. <https://doi.org/10.1016/j.meatsci.2004.10.007>